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Comparison of Estrogen, Progesterone and Her2 Receptors in Primary Breast Cancer and Paired Metastatic Lymph Nodes: An Immunohistochemical Study

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Abstract

Background: Immunohistochemistry (IHC) of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) are important prognostic factors of breast cancer. However, discordance between IHC findings of primary tumor and ipsilateral metastatic lymph nodes (IMLNs) has been reported that might affect the criteria for adjuvant therapies in breast cancer in the future.

Methods: Sample size of the current study was estimated 50 (Macnemar test). We performed IHC for ER, PR, and Her2 on IMLNs of 50 paraffin embedded blocks of breast cancer patients with regional lymphadenopathy during the period. All-red score 2 was regarded as negative and 3 as positive for ER/PR. Her2 results were classified to 1+ (0 and 1+), 2+, and 3+. We used SPSS 16 to insert and analyze the data.

Results: Mean age of patients was 49 yrs, and mean tumor size was 4.8 $\pm$ 3.53 cm. Twenty-four samples were pN1, 17 pN2, and 9 pN3. ER and PR were positive in 50% and 52 % of tumoral samples and 78% and 76% of IMLNs, respectively (fisher exact test, P = 0.003 and P = 0.011, respectively). The discrepancy between IHC of primary tumor and IMLNs for ER, PR and Her2 was 32% (P = 0.000), 24% (P = 0.002), and 48%, respectively. Overall, 34 patients (68%) showed disagreement in at least one of their receptors.

Conclusions: Discrepancy between IHC results of primary tumor and IMLNs was significant. Since metastatic clones in metastatic lymph nodes (MLNs) are potential sources of systemic metastasis, routine IHC on MLNs could play an important role in determining prognosis, indication for FISH, and finally, choosing adjuvant treatment.

Keywords: Breast Cancer, Lymphadenopathy, ER, PR, Her2

1. Background

Breast cancer is the most common cancer among women worldwide. A significant fraction of patients often die of metastatic disease. About 519000 women died in 2004 due to breast cancer. Although breast cancer is thought to be a disease of the developed world, a high percentage (69%) of all breast cancer deaths occurs in developing countries (1, 2). Breast cancer at early stages does not show any symptoms (1). This feature emphasizes the importance of regular breast exams. As the cancer grows, symptoms may include: breast lump, change in the size, shape or feel of the breast or nipple and fluid secretion from the nipple (3-6).

Breast cancer prognostic factors include axillary nodal status, clinical stage, size and grade of the tumor, hormone receptor status, and presence of lymphovascular involvement (7). Involvement of the regional lymph nodes is a major predictive factor of metastatic disease. Adjuvant therapies such as chemotherapy, radiotherapy, hormone therapy, and monoclonal antibodies reduce the incidence of metastasis greatly (8-10).

A predictive factor could be defined as any measurement related to the response to any given therapy. Prognostic factors play a key role in optimizing treatment for breast cancer patients as it leads to general use of adjuvant therapy (11). Estrogen (ER) and progesterone (PR) receptors, and human epidermal growth factor receptor 2 (HER2) are definitely listed as both prognostic and predictive factors (12-20). Hormone therapy and monoclonal antibodies are performed based on the results of immunohistochemistry (IHC) and / or immunofluorescence, which reveal corresponding receptors (estrogen, progesterone and Her2) in tumors (21-26). Although mentioned adjuvant therapies would not be routinely applied on metastatic lymph nodes, mismatch (qualitative and quan-

titative) of these receptors between the primary tumor and metastatic lymph nodes could result in secondary resistance to adjuvant treatments and occurrence of metastatic disease.

Even though several studies have noted the discrepancy between the immunohistochemistry findings of primary breast tumor and its metastases (27), there still is lack of enough studies comparing the results of primary breast tumor and lymphadenopathy immunohistochemistry. It seems that the receptor status of metastatic lymph nodes would be better criteria for administering adjuvant therapies. Also due to lack of sufficient studies in this field, we studied the qualitative evaluation of ER, PR and Her2 receptors in metastatic lymph nodes of breast cancer patients in Omid hospital of Mashhad from 2005 to 2010.

2. Methods

2.1. Patients

It was a cross sectional study and all the patients with involved regional lymph nodes (N +) were selected and a special check list was filled for them. We evaluated the health records of all the breast cancer patients who referred to our teaching hospital during five years (2005 - 2010).

Sampling method was simple non-possible, and sample size was calculated as 50 based on Mcnemar test and PASS software. Inclusion criteria were: all breast cancer patients who had axillary lymphadenopathy and Immunohistochemical process were performed on their tumors from 2005 to 2010. Exclusion criteria were: 1) unavailability of lymph node pathology samples, and 2) technical errors in staining procedures of lymph node pathology sample.

2.2. Method

Pathology samples of involved nodes were collected from the related laboratories and were assessed by Immunohistochemical markers (Skan Teb Asia) for ER, PR and Her2. The findings were compared with Immunohistochemical results of primary tumors and each change in percentage and staining was considered as discrepancy. We performed Immunohistochemistry for all the lymph node samples of patients upon whom IHC was done previously in the same center and with the same technique to prevent possible mistakes, comparing to the lymph node and tumor IHCs performed by one pathologist.

The interpretation of IHC findings was semi-qualitative, based on Red-score (from 1 to 8) for ER and PR, and each change from mentioned score in percentage or in staining rate was considered as discrepancy. IHC interpretation of Her2 was based on staining rate of cell membrane and it ranged from 0 to +3. All +3 cases were considered positive, 0 and +1cases were considered negative, and +2 cases were considered controversy and candidate for the Fish test. Finally, the gained findings were compared with primary tumor results.

2.3. Data Collection

The method of gathering data was library and check list form was the tool used in this study. Obtained data were analyzed by using SPSS 16.0 and Chi-Square MCnemar statistical test was performed in significant level of 0.05.

2.4. Ethical Consideration

We obtained consent from hospital officials and patients who participated in this study. Also patients were ensured about confidentiality of their recorded information and privacy. The code of the ethical consideration according to our office is IR.MUMS.REC.1390,40.

3. Results

The youngest patient was 27 years and the oldest was 77 years, and the mean age of patients was 48.02 years. Average size of tumor was 4.798 cm (Table 1).

Primary metastasis was positive for progesterone (PR) in 26 patients, for estrogen (ER) in 25 individuals, and for Her2 in 49 patients. Wilcoxon signed-rank test showed that there were significant differences between IHCs of primary tumor and metastasis for all three PR ($P < 0.001$), ER ($P < 0.001$) and Her2 ($P = 0.003$) (Table 2).

The comparison of primary tumor and lymphadenopathy immunohistochemistry showed that there was a significant difference in ER ($P = 0.003$) between them. ER was positive for primary tumor in 25 (50%) of the patients and it was positive for metastasis lymphadenopathy in 39 (78%) of individuals. Also chi-square test showed that there was a significant difference in PR between primary tumor and metastasis ($P = 0.011$). Positive PR was observed in 26 (52%) patients for primary tumor and it was seen in metastasis lymphadenopathy of 38 (76%) of patients (Table 3).

We compared the patients who had similar Her2 results in primary sample and metastasis with patients who had different findings for grade variables, LVST and, LN1-cod. Kruskal-Wallis test showed that there is no significant difference between these two groups ($P > 0.05$). Also we performed a comparison between patients with similar PR and ER results of primary tumor and metastatic lymphadenopathy, and patients with dissimilar findings. Kolmogorow-Smirnow-Test showed that there was no significant difference between these mentioned groups in ER and PR ($P > 0.05$).

Table 1. Age and Tumor Size in Patients with Breast Cancer

Variables	No.	Minimum	Maximum	Mean	Standard Deviation
Age	49	27	77	48.02	12.592
Size	46	1.0	17.0	4.798	3.5321

Table 2. IHCs of PRIMARY TUMOr and METASTasis in PR, ER and Her2 (Wilcoxon Signed Ranks Test)[a]

IHC	Positive	Negative	P Value, Wilcoxon Signed Ranks Test
PR	26 (52)	24 (48)	0.000
ER	25 (50)	25 (50)	0.000
Her2	49 (100)	-	0.003

[a]Values are expressed as No. (%).

T-test indicated that there was not any association between the above mentioned groups (those that had same results in tumor sample and metastatic lymphadenopathy, and those with different results) with variables such as age, tumor size, number of removed nodes and number of involved nodes. Also there was no significant difference in ER, PR and Her2 ($P > 0.05$). Twenty-four, seventeen and nine patients were pN1, pN2 and pN3, respectively.

Cohen's kappa coefficient was used to determine the association. Discrepancies of immunohistochemical ER, PR and Her2 results in primary tumor and lymphadenopathy were 24% ($P = 0.000$), 32% ($P = 0.002$), and 48%, respectively. Overall, a total of 34 patients (68%) had discrepancy for one of the receptors (Table 4).

4. Discussion

We evaluated the pathology samples of fifty patients who suffered from breast cancer.

All regional lymphadenopathy samples were tested by IHC for ER, PR and Her2 markers and finally we compared them with IHC findings of related primary tumors.

Atiken et al. (2009) studied the difference in expression of ER, PR and Her2 receptors between primary tumors and lymph nodes via immunofluorescence (28). Approximately in fifty percent of patients' samples of breast tumors and related nodes differed at least for one receptor. They claimed that considering lymph node receptor could be a more precise method for adjuvant treatment. Correspondingly, we showed that the difference between IHC of primary tumor and IMLNs for ER, PR and Her2 was 32% ($P = 0.000$), 24% ($P = 0.002$) and 48%, respectively.

Nedergaard assessed the ER receptor expression between primary tumor and metastatic lymph node (29). Discrepancy was observed for 21% of patients. They concluded that it was because of lack of ER receptors in metastatic cells and it could justify the failure of hormone therapy.

Cardoso et al. (2001) performed a similar study for the assessment of predictive biological makers (30). They showed that there is no marker with 100% association with both samples. They studied primary tumor and regional lymph nodes in the axillary area of the patients with breast cancer. For each marker, the percentage of stained cells (fatality) and its severity were assessed. IHC assessment was performed by using monoclonal antibodies against topo Π-alpha, Hsp27 (heat shock protein) HSP 70, HER2, p53 and bcl2.

Dissimilarly, Cho et al. (2008) showed that discrepancy was less than what we gained for expression of Her2 receptor between primary tumor and lymph node. They studied the marker expression status of HER 2, EGFR in primary tumor and lymph node metastasis in CYCLIN-D1 region (axillary) using IHC and CISH in 73 patients (31).

Although many studies indicate that Her2 and other hormone receptors play effective role in patient management therapy as we showed (32-34), there are researchers who believe that receptor status in recurrent tumors does not pose predictable value based on the analysis of hormone receptors in primary stage (27, 35, 36).

We showed significant differences between IHC findings of biomarkers in lymphadenopathy of primary tumor and metastatic node ($P < 0.05$). This could change the future of adjuvant treatments and the choice of best method for patients with cancer.

4.1. Conclusion

While the receptor status of metastatic lymph nodes would be better for selection of adjuvant therapies and there are not enough studies comparing the results of primary breast tumor and lymphadenopathy IHC, we decided to evaluate discrepancy between IHC results of primary tumor and ipsilateral metastatic lymph nodes (IMLNs). In our study it was significant. Since metastatic clones in MLNs are potential sources of systemic metastasis, routine IHC on MLNs could play an important role in determining prognosis, indication for FISH and finally choosing adjuvant treatment. The youngest patient was 27 years and the

Table 3. IHCs of Primary Tumor and Metastasis in PR, ER (Chi-Square Test)[a]

IHC	Primary Tumor		Metastasis Lymphadenopathy		P Value, Wilcoxon Signed Ranks Test
	Positive	Negative	Positive	Negative	
PR	26 (52)	24 (48)	38 (76)	12 (24)	0.011
ER	25 (50)	25 (50)	39 (78)	11 (22)	0.003

[a]Values are expressed as No. (%).

Table 4. Discrepancies of Immunohistochemical ER, PR and Her2 Results in Primary Tumor and Lymphadenopathy

IHC	Tumor	Lymph Node	No. (%)	Kappa Coefficient
ER	-	-	12 (24)	0.51*
	-	+	12 (24)	
	+	-	0 (0)	
	+	+	26 (52)	
PR	-	-	10 (20)	0.36*
	-	+	15 (30)	
	+	-	1 (2)	
	+	+	24 (48)	
Her2	+	+	13 (26.5)	0.253*
	+	++	11 (22.4)	
	+	+++	3 (6.1)	
	++	+	4 (8.2)	
	++	++	6 (12.2)	
	++	+++	5 (10.2)	
	+++	+	0 (0)	
	+++	++	1 (2)	
	+++	+++	6 (12.2)	

oldest was 77 years; mean age of patients was 48.02 years. Average size of tumor was 4.798 cm (Table 1).

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Footnotes

Authors' Contribution: None declared.

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References

1. World health organization . Available from: http://www.who.int/cancer/detection/breastcancer/en/index1.html.
2. World cancer research fund . Available from: http://www.wcrf.org/cancer_statistics/world_cancer_statistics.php.
3. Chalasani P, Downey L, Stopeck AT. Caring for the breast cancer survivor: a guide for primary care physicians. *Am J Med.* 2010;**123**(6):489–95. doi: 10.1016/j.amjmed.2009.09.042. [PubMed: 20569749].
4. Chlebowski RT, Anderson GL, Gass M, Lane DS, Aragaki AK, Kuller LH, et al. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. *JAMA.* 2010;**304**(15):1684–92. doi: 10.1001/jama.2010.1500. [PubMed: 20959578].
5. Chlebowski RT, Kuller LH, Prentice RL, Stefanick ML, Manson JE, Gass M, et al. Breast cancer after use of estrogen plus progestin in postmenopausal women. *N Engl J Med.* 2009;**360**(6):573–87. doi: 10.1056/NEJMoa0807684. [PubMed: 19196674].
6. Cuzick J, DeCensi A, Arun B, Brown PH, Castiglione M, Dunn B, et al. Preventive therapy for breast cancer: a consensus statement. *Lancet Oncol.* 2011;**12**(5):496–503. doi: 10.1016/S1470-2045(11)70030-4. [PubMed: 21441069].
7. Stearns V, Hayes DF, editors. New prognostic factors for breast cancer recurrence breast cancer program. Lombardi Cancer Center. Georgetown University, Washington, DC. .
8. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. *Oncologist.* 2004;**9**(6):606–16. doi: 10.1634/theoncologist.9-6-606. [PubMed: 15561805].
9. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet.* 1998;**351**(9114):1451–67. doi: 10.1016/S0140-6736(97)11423-4. [PubMed: 9605801].
10. Polychemotherapy for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet.* 1998;**352**(9132):930–42. [PubMed: 9752815].
11. Clark GM. Do we really need prognostic factors for breast cancer?. *Breast Cancer Res Treat.* 1994;**30**(2):117–26. doi: 10.1007/BF00666054. [PubMed: 7949209].

12. Fisher B, Redmond C, Fisher ER, Caplan R. Relative worth of estrogen or progesterone receptor and pathologic characteristics of differentiation as indicators of prognosis in node negative breast cancer patients: findings from National Surgical Adjuvant Breast and Bowel Project Protocol B-06. *J Clin Oncol.* 1988;**6**(7):1076–87. doi: 10.1200/JCO.1988.6.7.1076. [PubMed: 2856862].
13. Hilsenbeck SG, Ravdin PM, de Moor CA, Chamness GC, Osborne CK, Clark GM. Time-dependence of hazard ratios for prognostic factors in primary breast cancer. *Breast Cancer Res Treat.* 1998;**52**(1-3):227–37. doi: 10.1023/A:1006133418245. [PubMed: 10066085].
14. Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature.* 1984;**312**(5994):513–6. doi: 10.1038/312513a0. [PubMed: 6095109].
15. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science.* 1987;**235**(4785):177–82. doi: 10.1126/science.3798106. [PubMed: 3798106].
16. Borg A, Tandon AK, Sigurdsson H, Clark GM, Ferno M, Fuqua SA, et al. HER-2/neu amplification predicts poor survival in node-positive breast cancer. *Cancer Res.* 1990;**50**(14):4332–7. [PubMed: 1973070].
17. Winstanley J, Cooke T, Murray GD, Platt-Higgins A, George WD, Holt S, et al. The long term prognostic significance of c-erbB-2 in primary breast cancer. *Br J Cancer.* 1991;**63**(3):447–50. doi: 10.1038/bjc.1991.103. [PubMed: 1672256].
18. Paterson MC, Dietrich KD, Danyluk J, Paterson AH, Lees AW, Jamil N, et al. Correlation between c-erbB-2 amplification and risk of recurrent disease in node-negative breast cancer. *Cancer Res.* 1991;**51**(2):556–67. [PubMed: 1670762].
19. Clark GM, McGuire WL. Follow-up study of HER-2/neu amplification in primary breast cancer. *Cancer Res.* 1991;**51**(3):944–8. [PubMed: 1988136].
20. Ravdin PM, Green S, Albain KS. Initial report of the SWOG biological correlative study of c-erb-2 expression as a predictor of outcome in a trial comparing adjuvant CAF T with tamoxifen (T) alone. *Proc Am Soc Clin Oncol.* 1998;**17**:97.
21. McCarty KJ, Miller LS, Cox EB, Konrath J, McCarty KS. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med.* 1985;**109**(8):716–21. [PubMed: 3893381].
22. Barnes DM, Harris WH, Smith P, Millis RR, Rubens RD. Immunohistochemical determination of oestrogen receptor: comparison of different methods of assessment of staining and correlation with clinical outcome of breast cancer patients. *Br J Cancer.* 1996;**74**(9):1445–51. doi: 10.1038/bjc.1996.563. [PubMed: 8912543].
23. Rastelli F, Crispino S. Factors predictive of response to hormone therapy in breast cancer. *Tumori.* 2008;**94**(3):370–83. [PubMed: 18705406].
24. Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, et al. Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immuno-histochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study. *Int J Cancer.* 2000;**89**(2):111–7. doi: 10.1002/(SICI)1097-0215(20000320)89:2<111::AID-IJC2>3.3.CO;2-N. [PubMed: 10754487].
25. Rexhepaj E, Brennan DJ, Holloway P, Kay EW, McCann AH, Landberg G, et al. Novel image analysis approach for quantifying expression of nuclear proteins assessed by immunohistochemistry: application to measurement of oestrogen and progesterone receptor levels in breast cancer. *Breast Cancer Res.* 2008;**10**(5):R89. doi: 10.1186/bcr2187. [PubMed: 18947395].
26. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol.* 1999;**17**(5):1474–81. doi: 10.1200/JCO.1999.17.5.1474. [PubMed: 10334533].
27. Saeedi Saedi H, Ghavam Nasiri MR, ShahidSales S, Taghizadeh A, Mohammadian N. Comparison of hormone receptor status in primary and recurrent breast cancer. *Iran J Cancer Prev.* 2012;**5**(2):69–73. [PubMed: 25628823].
28. Aitken SJ, Thomas JS, Langdon SP, Harrison DJ, Faratian D. Quantitative analysis of changes in ER, PR and HER2 expression in primary breast cancer and paired nodal metastases. *Ann Oncol.* 2010;**21**(6):1254–61. doi: 10.1093/annonc/mdp427. [PubMed: 19858088].
29. Nedergaard L, Haerslev T, Jacobsen GK. Immunohistochemical study of estrogen receptors in primary breast carcinomas and their lymph node metastases including comparison of two monoclonal antibodies. *APMIS.* 1995;**103**(1):20–4. [PubMed: 7695887].
30. Cardoso F, Di Leo A, Larsimont D, Gancberg D, Rouas G, Dolci S, et al. Evaluation of HER2, p53, bcl-2, topoisomerase II-alpha, heat shock proteins 27 and 70 in primary breast cancer and metastatic ipsilateral axillary lymph nodes. *Ann Oncol.* 2001;**12**(5):615–20. [PubMed: 11432618].
31. Cho EY, Han JJ, Choi YL, Kim KM, Oh YL. Comparison of Her-2, EGFR and cyclin D1 in primary breast cancer and paired metastatic lymph nodes: an immunohistochemical and chromogenic in situ hybridization study. *J Korean Med Sci.* 2008;**23**(6):1053–61. doi: 10.3346/jkms.2008.23.6.1053. [PubMed: 19119452].
32. Payne SJ, Bowen RL, Jones JL, Wells CA. Predictive markers in breast cancer–the present. *Histopathology.* 2008;**52**(1):82–90. doi: 10.1111/j.1365-2559.2007.02897.x. [PubMed: 18171419].
33. Tiwari DK, Tanaka S, Inouye Y, Yoshizawa K, Watanabe TM, Jin T. Synthesis and Characterization of Anti-HER2 Antibody Conjugated CdSe/CdZnS Quantum Dots for Fluorescence Imaging of Breast Cancer Cells. *Sensors (Basel).* 2009;**9**(11):9332–64. doi: 10.3390/s91109332. [PubMed: 22291567].
34. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat Biotechnol.* 2003;**21**(1):41–6. doi: 10.1038/nbt764. [PubMed: 12459735].
35. Simmons C, Miller N, Geddie W, Gianfelice D, Oldfield M, Dranitsaris G, et al. Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases?. *Ann Oncol.* 2009;**20**(9):1499–504. doi: 10.1093/annonc/mdp028. [PubMed: 19299408].
36. Elledge RM, Fuqua SA. In: Estrogen and progesterone receptors. Harris JR, editor. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 471.